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Marked hyperleptinemia after high-fat diet associated with severe glucose intolerance in mice

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Abstract

We asked whether the likelihood for mice of the C57BL/6J strain to develop glucose intolerance when fed a high-fat diet is related to the increase in circulating levels of leptin or free fatty acids (FFA). We therefore administered a high-fat diet (58% fat) or a control diet (11% fat) for 1.5 years. NMRI mice were used as a more glucose-tolerant control group. After a high-fat diet, the area under the glucose curve following an intraperitoneal glucose challenge (1 g/kg) increased more markedly in C57BL/6J mice (by 42 ± 8%) than in NMRI mice (by 21 ± 3%, $P = 0.007$). Plasma levels of insulin, leptin and FFA increased in both strains of mice, whereas plasma glucose levels were elevated after the high-fat diet only in C57BL/6J mice. The slope of the relationship between body weight and plasma leptin was higher in C57BL/6J mice than in NMRI mice, suggesting leptin insensitivity. Circulating leptin correlated to circulating insulin in both strains of mice, whereas plasma FFA correlated to plasma insulin in NMRI mice but not in C57BL/6J mice. These correlations remained significant after adjustment for body weight. The results show that elevated leptin and FFA levels evolve after high-fat feeding in mice, in conjunction with evolvement of glucose intolerance and hyperglycemia.

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Introduction

Obesity is associated with insulin resistance, which is sensed by the islet B cells causing increased insulin secretion (1–3). The ensuing hyperinsulinemia is usually adequate for preventing hyperglycemia. However, if the signals to stimulate insulin secretion fail, the hyperinsulinemia might be inadequate, which could result in glucose intolerance or diabetes. Therefore, knowledge of the signals between insulin resistance and insulin secretion is of importance for the understanding of diabetes pathogenesis. To generate such knowledge, animal models have been developed (4). One well characterized model is the C57BL/6J mouse, which is the background strain for the ob/ob mutation (5). When given a high-fat diet, C57BL/6J mice develop obesity, hyperglycemia, hyperinsulinemia and impaired glucose-stimulated insulin secretion (6–9). The tendency for C57BL/6J mice to develop glucose intolerance upon feeding a high-fat diet is in contrast to other strains of mice, for example the A/J mouse (8).

It has previously been demonstrated that circulating levels of both leptin and lipids are increased upon high-fat feeding of C57BL/6J mice (6, 10, 11). Whether these responses are of importance for the compensatory changes in islet function accompanying the high-fat feeding or whether the responses are involved in the development of glucose intolerance, which follows high-fat feeding in C57BL/6J mice, is not known. In this study, we therefore asked whether the likelihood for C57BL/6J mice to develop glucose intolerance when fed a high-fat diet is related to the increase in circulating levels of leptin or free fatty acids (FFA). We gave a high-fat diet to both C57BL/6J mice and normal NMRI mice (which evolve only a marginal insulin resistance with a low degree of glucose intolerance on a high-fat diet) and analyzed plasma levels of leptin and FFA and related these parameters to body weight and the circulating levels of insulin and glucose.

Methods

Animals

Mice of the C57BL/6J strain or the NMRI strain were obtained from Bomholtgaard Breeding and Research Centre, Ry, Denmark, at 4 weeks of age. All mice were females, to avoid the profound gender differences in circulating leptin, which has been documented in humans. Half of the mice in each batch received a high-fat diet (Research Diets, N Brunswick, NJ, USA),

whereas the other half of the animals received an ordinary rodent chow diet (Lactamin AB, Stockholm, Sweden). On a caloric base, the high-fat diet consisted of 16.4% protein, 25.6% carbohydrates and 58.0% fat (total 23.4 kJ/g), whereas the control diet consisted of 25.8% protein, 62.8% carbohydrates and 11.4% fat (total 12.6 kJ/g). Throughout the study period, the mice had free access to food and water. Four to five mice were kept per cage in a temperature-controlled (22 ± 1 °C) room with a 12 h light:12 h darkness cycle with lights on at 0600 h. The experiments were undertaken after 1.5 years on the respective diet, at a time point where the difference in the development of obesity and glucose intolerance in the C57BL/6J mice as compared with NMRI mice is large and stable. The study was approved by the Animal Ethics Committee at Lund University.

Experiments

After 1.5 years on the respective diet, 68 non-fasted mice (13 C57BL/6J high-fat diet, 15 C57BL/6J control diet, 20 NMRI high-fat diet, 20 NMRI control diet) were injected intraperitoneally (i.p.) with D-glucose (Fluka Chemie AG, Buchs, Switzerland) at 1 g/kg. The volume load was 10 μl/g body weight. Blood samples were taken immediately before the glucose challenge and after 10, 30, 60 and 120 min. After centrifugation, plasma was stored at −20 °C until assayed for glucose concentration.

Blood sampling

After 1.5 years on high-fat or control diets, a blood sample was taken from 163 non-fasted animals (46 C57BL/6J high-fat diet, 48 C57BL/6J control diet, 34 NMRI high-fat diet, 35 NMRI control diet). The samples were taken from the intraorbital, retrobulbar plexus for the measurement of plasma levels of glucose, insulin, leptin and FFA in non-fasted animals. After centrifugation, plasma was stored at −20 °C until assayed. Simultaneously, body weight was determined.

Analyses

Plasma leptin levels were determined with a newly developed RIA specific for mouse leptin (10) (Linco Research Inc., St Charles, MO, USA). The method uses a polyclonal rabbit antibody raised against highly purified recombinant mouse leptin, ^{125}I-labeled tracer prepared with recombinant mouse leptin and mouse leptin as standard. Anti-rabbit IgG was used for separation of bound and free leptin. Coefficients of variations (CV) ranged from 4.0 to 11.2% within runs and from 3.3 to 14.6% between runs. Plasma insulin was determined radioimmunochemically with the use of a guinea pig anti-rat insulin antibody, ^{125}I-labelled porcine insulin as tracer and rat insulin as standard (Linco). Free and bound radioactivity were separated by use of an anti-IgG (goat anti-guinea pig) antibody (Linco). The sensitivity of the assay is 12 pmol/l and the CV less than 3% at both low and high levels. Glucose was determined with the glucose oxidase method, and FFA were extracted and measured photometrically (12).

Statistics

Means ± S.E.M. are shown. Statistical analyses were performed with the SPSS for Windows system. Statistical comparisons for the differences between high-fat and control diet treated mice were performed by Student's unpaired *t*-test. Pearson's product moment correlation was used to estimate linear relationships between variables. An insulin resistance index (IR_I) was calculated as plasma insulin × plasma glucose in each individual mouse. This index has been verified as a substitute for hyperinsulinemic clamp studies in humans to quantify insulin sensitivity (13, 14) and has recently been used as a crude estimation for insulin sensitivity in studies in mice (15).

Results

Glucose tolerance test

The i.p. glucose tolerance test showed that in both strains of mice, high-fat diet induced glucose intolerance. Individual glucose levels were significantly higher in high-fat diet fed at 30 and 60 min compared with the respective control mice (Fig. 1). At 120 min after glucose challenge, plasma glucose was still markedly higher in high-fat diet fed C57BL/6J mice (24.9 ± 3.2 mmol/l) compared with the controls (14.3 ± 1.7 mmol/l, $P = 0.011$), whereas in NMRI mice, plasma glucose levels at 120 min were not significantly different between the two groups. Also, the area under the curve for glucose ($AUC_{glucose}$) was increased by high-fat diet in both strains. In C57BL/6J mice given high-fat diet, $AUC_{glucose}$ was 2.5 ± 0.2 mol/l × 120 min vs 1.8 ± 0.2 mol/l × 120 min in mice given the control diet ($P = 0.025$), and the corresponding figures in NMRI mice were 1.0 ± 0.1 vs 0.8 ± 0.08 mol/l × 120 min ($P < 0.008$). The worsening of glucose tolerance was more marked in C57BL/6J mice than in NMRI mice after high-fat diet, since $AUC_{glucose}$ was increased by 42 ± 8% in C57BL/6J mice vs by 21 ± 3% in NMRI mice ($P = 0.007$).

Body weight and plasma levels of glucose, insulin, leptin and FFA

High-fat diet increased body weight in both strains of mice. Also plasma levels of insulin, leptin and FFA increased in both strains of mice, whereas plasma glucose levels were significantly elevated after high-fat

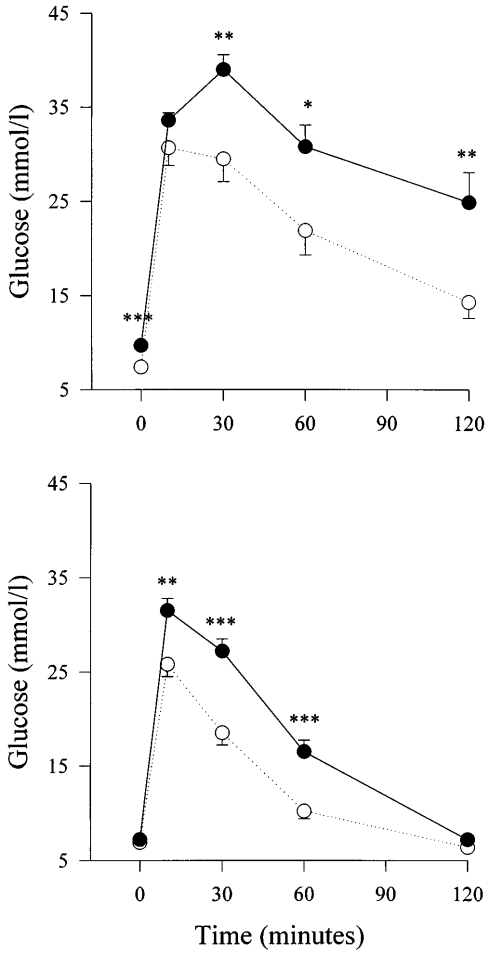

Figure 1 Plasma levels of glucose before and after an i.p. injection of glucose (1 g/kg) in C57BL/6J mice (upper panel) or NMRI mice (lower panel) given high-fat diet (●) or control diet (○) for 1.5 years. The number of animals in each group was: C57BL/6J high-fat diet 15, C57BL/6J control diet 13, NMRI high-fat diet 20, NMRI control diet 20. Means ± S.E.M. are shown. Asterisks indicate probability level of random difference between the control and the high-fat diet groups, *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

diet only in C57BL/6J mice and not in NMRI mice. Similarly, the calculated IR_I was elevated in both strains of mice (Table 1).

Correlation between body weight and plasma leptin and insulin

There was a close correlation between body weight and plasma leptin in both strains of mice (Fig. 2). The slope of the relationship between body weight and leptin was different in different subgroups of the mice. Thus, the slope was higher in C57BL/6J mice than in NMRI mice ($P < 0.001$). Furthermore, among the C57BL/6J mice, the slope of the relationship was considerably higher in the mice given high-fat diet than in mice given the control diet ($P < 0.001$; Table 2). This resulted in a

Table 1 Body weight, plasma levels of glucose, insulin, leptin and FFA and IR_I (means ± S.E.M.) in C57BL/6J and NMRI mice fed a high-fat diet or a control diet for 1.5 years.

	C57BL/6J mice				NMRI mice			
Parameter	High-fat diet ($n = 46$)	Control diet ($n = 48$)	P	Percent increase of mean value by high-fat diet	High-fat diet ($n = 35$)	Control diet ($n = 34$)	P	Percent increase of mean value by high-fat diet
Body weight (g)	32.9 ± 0.7	25.2 ± 0.3	<0.001	30	41.1 ± 1.2	35.8 ± 0.6	<0.001	17
Plasma glucose (mmol)	7.5 ± 0.1	6.9 ± 0.1	0.004	8	6.9 ± 0.2	6.8 ± 0.2	0.840	NS
Plasma insulin (pmol/l)	490 ± 36	202 ± 20	<0.001	142	435 ± 51	236 ± 44	0.026	84
Plasma leptin (ng/ml)	50.4 ± 2.8	9.8 ± 0.5	<0.001	412	22.2 ± 1.7	8.0 ± 0.7	<0.001	175
Ratio plasma leptin to body weight (ng/ml/g)	1.49 ± 0.07	0.38 ± 0.02	<0.001	292	0.53 ± 0.04	0.22 ± 0.02	<0.001	140
Plasma FFA (meq/l)	1.72 ± 0.07	1.31 ± 0.06	<0.001	32	1.91 ± 0.06	1.69 ± 0.09	0.041	13
IR_I (10^{-3} pmol insulin × mmol glucose × 1^{-2})	3.7 ± 0.3	1.4 ± 0.2	<0.001	164	3.0 ± 0.4	1.6 ± 0.3	0.004	87

P indicates the probability level of random difference (NS indicates not significant).

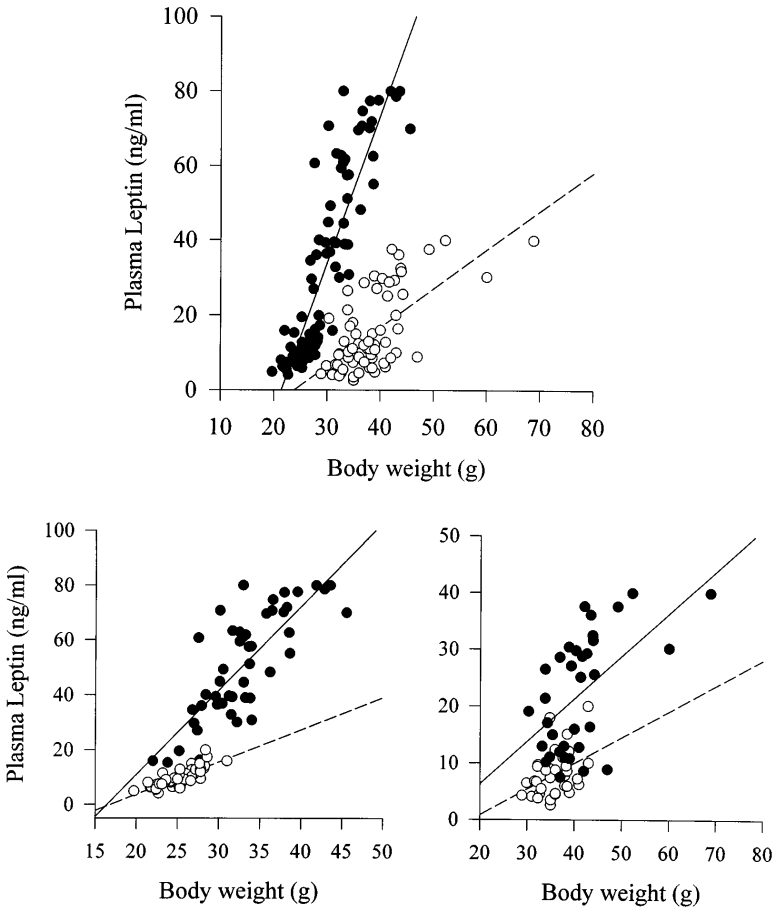

Figure 2 Upper panel: relationship between plasma leptin levels and body weight in C57BL/6J (●) and NMRI mice (○) given high-fat diet or control diet for 1.5 years (i.e. figures show the relationship in all animals irrespective of diet). Lower panels: relationship between plasma leptin levels and body weight in C57BL/6J (left panel) and NMRI mice (right panel) given high-fat diet (●) or control diet (○) for 1.5 years (i.e. in respective diets in the respective two strains). The number of animals in each group was: C57BL/6J mice 94 (high-fat diet 46, control diet 48), NMRI mice 69 (high-fat diet 35, control diet 34).

higher plasma leptin to body weight ratio in high-fat diet fed mice than in control diet fed mice, and a particularly high plasma leptin to body weight ratio in high-fat diet fed C57BL/6J mice (Table 1).

Correlation between plasma leptin, plasma insulin, insulin resistance and plasma FFA

In both strains of mice, plasma leptin correlated to plasma insulin (C57BL/6J mice $r = 0.55$, $P < 0.001$; NMRI mice $r = 0.41$, $P = 0.012$) and to the IR_I (C57BL/6J mice $r = 0.60$, $P < 0.001$; NMRI mice $r = 0.37$, $P = 0.002$). In C57BL/6J mice, these correlations remained significant after adjustment for the influence of body weight, since the ratio of plasma leptin to body weight correlated to the ratio of plasma insulin to body weight ($r = 0.38$, $P < 0.001$) as well as to the ratio of IR_I to body weight ($r = 0.45$, $P < 0.001$). Similarly, plasma leptin correlated to plasma insulin independently of body weight also, because in a partial correlation

Table 2 Relationship between body weight and plasma levels of leptin in C57BL/6J and NMRI mice fed a high-fat diet or a control diet for 1.5 years.

	Coefficient of the relationship (*r*) between body weight and plasma leptin	Slope of the relationship between body weight and plasma leptin (ng/ml/g ± S.E.M.)
C57BL/6J (both diets, $n = 94$)	0.88 ($P < 0.001$)	3.98 ± 0.22
C57BL/6J (high fat diet, $n = 46$)	0.79 ($P < 0.001$)	3.06 ± 0.35
C57BL/6J (control diet, $n = 48$)	0.80 ($P < 0.001$)	1.18 ± 0.13
NMRI (both diets, $n = 69$)	0.64 ($P < 0.001$)	1.04 ± 0.15
NMRI (high-fat diet, $n = 35$)	0.55 ($P = 0.001$)	0.75 ± 0.19
NMRI (control diet, $n = 34$)	0.41 ($P = 0.017$)	0.45 ± 0.18

P indicates probability level of the regression.

analysis these parameters correlated significantly after controlling for the influence of body weight ($r = 0.41$, $P < 0.001$). In NMRI mice, the ratio of leptin to body weight correlated to the ratio of plasma insulin to body weight ($r = 0.30$, $P = 0.038$), but not to the ratio of IR_I to body weight ($r = 0.19$, $P = 0.116$). As in the case of C57BL/6J mice, plasma leptin correlated to plasma insulin independently of body weight also in NMRI mice because in a partial correlation analysis these parameters correlated significantly after controlling for the influence of body weight ($r = 0.28$, $P = 0.045$). Plasma leptin correlated markedly to plasma FFA in C57BL/6J mice ($r = 0.52$, $P < 0.001$) but only marginally in NMRI mice ($r = 0.25$, $P = 0.049$). However, after adjustment for body weight, these correlations were not significant (C57BL/6J mice $r = 0.04$, $P = 0.687$, NMRI mice $r = -0.11$, $P = 0.373$).

Correlation between plasma FFA and insulin resistance

In C57BL/6J mice, plasma FFA correlated to body weight ($r = 0.37$, $P < 0.001$), plasma insulin ($r = 0.39$, $P < 0.001$) and IR_I ($r = 0.34$, $P = 0.001$). The correlation between plasma FFA and IR_I remained significant after adjustment for body weight ($r = 0.28$, $P = 0.039$), whereas after adjustment for body weight, the correlation between plasma FFA and plasma insulin was no longer significant. In NMRI mice, plasma FFA correlated to plasma insulin ($r = 0.46$, $P = 0.008$) and IR_I ($r = 0.41$, $P = 0.012$), and both these correlations remained significant after adjustment for body weight ($r = 0.42$, $P = 0.016$ for insulin, $r = 0.44$, $P = 0.009$ for IR_I).

Discussion

High-fat diet is known to induce insulin resistance in rodents (6, 7, 16, 17). In this study, both strains of mice examined (C57BL/6J and NMRI) developed hyperinsulinemia following 1.5 years of feeding a high-fat diet. Furthermore, the IR_I was increased after the high-fat diet in both strains. Therefore, both C57BL/6J and NMRI mice develop insulin resistance after the high-fat diet. Both basal plasma insulin and IR_I were similar in the two strains of mice after the high-fat diet, suggesting that a similar degree of insulin resistance had been induced. However, the metabolic consequences of the insulin resistance seem to differ between the two strains. In the NMRI mice, the evolving hyperinsulinemia was adequate for maintaining normoglycemia, since plasma glucose levels were not increased by the high-fat diet. In contrast, in C57BL/6J mice, a slight hyperglycemia evolved in spite of the marked hyperinsulinemia, indicating inadequate hyperinsulinemia. Consequently, glucose tolerance, as judged by an i.p. glucose tolerance test, had deteriorated more severely in the C57BL/6J than in the NMRI mice.

This study examined whether plasma leptin and/or FFA correlated to the different sensitivity to high-fat diet in the two strains. Both strains of mice evolved hyperleptinemia in response to the high-fat diet, which confirms previous reports (10, 11). We here also demonstrate that C57BL/6J mice exhibited a more marked increase in plasma leptin during the 1.5 years of feeding with the high-fat diet than NMRI mice. The C57BL/6J mice developed a higher leptin to body weight ratio than the NMRI mice after high-fat diet. Although body composition was not determined in the mice, the higher leptin to body weight ratio in C57BL/6J mice might indicate a reduced leptin sensitivity in this strain of mice. This confirms a previous study demonstrating that plasma leptin in C57BL/6J mice given a high-fat diet on a long-term basis is higher than in another strain of mice being not as glucose intolerant (11). This suggests that mice developing the most marked glucose intolerance on the high-fat diet also exhibit the lowest leptin sensitivity.

We found that in both C57BL/6J and NMRI mice, plasma leptin correlated to plasma insulin. This is a well known relationship in both mice (10) and humans (18, 19). Such a correlation might be explained by insulin stimulating leptin production, which is known as a phenomenon executed at the adipocyte level (20). However, it might also be suggested that leptin stimulates insulin secretion, and therefore supports the hyperinsulinemia in insulin resistance. Previously observed correlations between plasma leptin and indices of insulin secretion are suggestive of this notion (18, 21), as are studies demonstrating that leptin stimulates insulin secretion from insulin-producing cells (22, 23). This topic is controversial, however, since most studies have demonstrated an inhibition by leptin of insulin secretion (24–26). We found that plasma leptin correlated to IR_I in both strains of mice. In the C57BL/6J mice, this relationship was independent of body weight. Previous studies in humans on the relationship between circulating leptin and insulin sensitivity have not been consistent, probably due to different groups of subjects under study (18, 27, 28).

FFA have been suggested to be involved in the mediation of hyperinsulinemia in insulin resistance, since plasma levels of FFA are increased in obesity (29) and FFA are known to stimulate insulin secretion (30). Following a long-term stimulation of the B cells, lipotoxicity might evolve, inhibiting insulin secretion (3). This might cause islet dysfunction and impaired glucose tolerance. Our present results support such a notion, since plasma FFA correlated to insulin after adjustment for body weight in NMRI mice but not in C57BL/6J mice. This would suggest that FFA support insulin secretion in NMRI mice whereas in C57BL/6J mice, FFA failed to adequately support insulin secretion yielding inadequate hyperinsulinemia, which was then followed by hyperglycemia and glucose intolerance. The slight hyperglycemia then further increases plasma

insulin, explaining why C57BL/6J mice did not have lower plasma insulin than NMRI mice. The results therefore suggest that the lipotoxicity (illustrated by the lack of correlation between plasma FFA and insulin in C57BL/6J mice) prevents the degree of hyperinsulinemia which is required for maintaining normoglycemia. Since at the same time, C57BL/6J mice seemed to be leptin resistant, it is tempting to speculate that a function of leptin is to counteract the lipotoxic action of FFA, which is a hypothesis in line with recent suggestions by Unger (3). This would mean that in NMRI mice, leptin might have prevented the lipotoxic action of FFA allowing normoglycemia to persist, whereas in C57BL/6J mice leptin fails to do this due to leptin resistance. In line with this hypothesis, a recent study using hyperleptinemic rats has shown that chronically elevating plasma leptin increases insulin secretion and proinsulin synthesis in obese rats (15).

In both strains of mice there was a relationship between plasma leptin and plasma FFA. However, after adjustment for body weight, no significant correlations were evident between these parameters, indicating that they both increased due to the increase in body weight. A previous study has also failed to detect any direct relationship between circulating leptin and FFA (31).

This study has focused on the long-term effects of a high-fat diet. It is impossible to establish the mechanism underlying the induction of insulin resistance from the results of this study, since hyperinsulinemia and hyperglycemia, i.e. signs of insulin resistance, occur very early after introducing a high-fat diet (6). Both leptin and FFA might be the mediators, since it has been shown that FFA inhibit glucose oxidation and glycolysis (32, 33) and leptin has been shown to counteract the action of insulin (34, 35). However, the potential role of leptin in this regard is not yet resolved, since leptin also has been demonstrated to augment insulin action (34, 36).

In conclusion, this study has shown that 1.5 years of high-fat diet increases plasma leptin and FFA in two different strains of mice. Furthermore, the high-fat diet results in a more pronounced glucose intolerance in one strain, C57BL/6J, than in another, NMRI. This difference is accompanied by a slight hyperglycemia in C57BL/6J mice, which is not seen in NMRI mice. The marked impairment of glucose tolerance in C57BL/6J mice is accompanied by a higher plasma leptin in relation to body weight, a sign of leptin resistance. Furthermore, in glucose-intolerance prone C57BL/6J mice, FFA did not correlate to basal insulin, whereas in NMRI mice, such a correlation was significant. The results are in line with a hypothesis that in insulin resistance, plasma FFA stimulate insulin secretion to support the hyperinsulinemia for the avoidance of hyperglycemia, and that leptin counteracts the long-term development of lipotoxicity. Consequently, if leptin resistance evolves, the lipotoxic action of FFA becomes unopposed, and glucose intolerance evolves.

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